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Litter decomposition can be reduced by pesticide effects on detritivores and decomposers: Implications for tropical stream functioning☆

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ABSTRACT

Understanding which factors affect the process of leaf litter decomposition is crucial if we are to predict changes in the functioning of stream ecosystems as a result of human activities. One major activity with known consequences on streams is agriculture, which is of particular concern in tropical regions, where forests are being rapidly replaced by crops. While pesticides are potential drivers of reduced decomposition rates observed in agricultural tropical streams, their specific effects on the performance of decomposers and detritivores are mostly unknown. We used a microcosm experiment to examine the individual and joint effects of an insecticide (chlorpyrifos) and a fungicide (chlorothalonil) on survival and growth of detritivores (*Anchytarsus, Hyalella* and *Lepidostoma*), aquatic hyphomycetes (AH) sporulation rate, taxon richness, assemblage structure, and leaf litter decomposition rates. Our results revealed detrimental effects on detritivore survival (which were mostly due to the insecticide and strongest for *Hyalella*), changes in AH assemblage structure, and reduced sporulation rate, taxon richness and microbial decomposition (mostly in response to the fungicide). Total decomposition was reduced especially when the pesticides were combined, suggesting that they operated differently and their effects were additive. Importantly, effects on decomposition were greater for single-species detritivore treatments than for the 3-species mixture, indicating that detritivore species loss may exacerbate the consequences of pesticides of stream ecosystem functioning.

1. Introduction

Riverine ecosystems are highly vulnerable to biodiversity loss and changes in ecosystem functioning due to a wide range of anthropogenic stressors (Chauvet et al., 2016; Reid et al., 2018). Of the human activities that have detrimental impact on streams, one of the strongest is agriculture, which is particularly concerning in tropical regions of the world, where forests are rapidly being replaced by crops (Gibbs et al., 2010). Agriculture involves a number of stressors, including habitat loss (Burdon et al., 2013), organic pollution (Graeber et al., 2015) and the presence of pesticides (Schafer et al., 2007), all of which can interact to impair tropical stream communities (Cornejo et al., 2019) and ecosystem functioning (Cornejo et al., 2020b). However, there is little information about the specific effects that pesticides have on key ecosystem processes such as leaf litter decomposition, and the organisms mediating this process (i.e., microbial decomposers and litter-feeding detritivores), in the tropics.

The process of leaf litter decomposition is a key component of stream ecosystem functioning, often used as indicator of ecosystem integrity (Gessner and Chauvet, 2002; Young et al., 2008) and relevant for global biogeochemical cycles (Boyero et al., 2011b; Handa et al., 2014). Understanding which factors affect decomposition is thus crucial, but it is complex because the process involves multiple biological interactions at different trophic levels (Gessner

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et al., 2010). The identity and diversity of leaf litter (López-Rojo et al., 2020), microbial decomposers (Santschi et al., 2017) and detritivores (Boyero et al., 2014) can all influence decomposition. Moreover, different species, numbers and combinations of species can be differently affected by particular stressors (Maltby, 1999; Thompson et al., 2018), and thus modulate their effects on decomposition.

Effects of pesticides on microbial decomposers and detritivores in the tropics are mostly unknown. However, experiments conducted in other areas of the world suggest that these organisms are probably impaired by pesticides, and these effects are likely to reduce litter decomposition rates (Zubrod et al., 2014; Cornejo et al., 2020a). Another unexplored question is whether more diverse detritivore assemblages attenuate the effects of pesticides on decomposition. This is possible because diversity increases the number of functional traits present in the assemblage (Petchey and Gaston, 2006), possibly including a broader range of sensitivity to stressors (Ippolito et al., 2012). This issue is particularly important for tropical streams because they often have low detritivore diversity locally, even when the regional species pool is high (Boyero et al., 2011a, 2012, 2015), so pesticides may therefore have greater effects on decomposition in tropical than in temperate streams.

We examined the above questions in a microcosm experiment using two pesticides that are among the most widely used in agriculture in our study region (western Panama), where they have been found at concentrations that exceed the recommended levels (MIDA, 2016): the insecticide chlorpyrifos and the fungicide chlorothalonil. We explored how these two pesticides, individually and together, at low and high concentrations in both cases, impacted (i) three common detritivores in the study area, individually and together (i.e., in 1-species and 3-species assemblages, respectively); (ii) assemblages of microbial decomposers (i.e., aquatic hyphomycetes, hereafter AH), and (iii) decomposition rates (total and microbial). We tested the following hypotheses: (1) the pesticides reduce detritivore survival and growth, AH sporulation rate and taxon richness, and decomposition rates, and alter AH assemblage structure; (2) effects of each pesticide are greater at higher concentration and when they are in combination; and (3) pesticide effects on different detritivore species follow a similar trajectory but vary in intensity (due to differences in species sensitivity), with effects lower in the 3-species detritivore assemblage compared to single species.

2. Materials and methods

2.1. Study area

The microcosm experiment was conducted in November and December 2019 (wet season) at the outdoor facilities of the Ministry of the Environment Station in La Amistad International Park (PILA; 88.94 °N, 82.615 °W; Fig. S1). The PILA station is located ca. 400 m from the headwater stream of the Chiriquí Viejo River upper catchment, in the Pacific coast of western Panama (8.25–9.00 °N, 82.25–83.00 °W). The area has a tropical climate with minimum, average and maximum air temperatures of 17.8, 28.0 and 35.5 °C, respectively (ANAM, 2014). Total average annual precipitation is 3400 mm, with a maximum of 7000 mm at high altitudes, of which 87.7% occurs in the wet season from May to December (ETESA, 2008).

2.2. Pesticides

We used two of the pesticides most commonly used in the upper catchment of the Chiriquí Viejo river (Cornejo et al., 2019, 2020b) in the experiment: the insecticide chlorpyrifos and the fungicide chlorothalonil. Chlorpyrifos ($C_9H_{11}Cl_3NO_3PS$) is an organophosphate insecticide that inhibits acetylcholinesterase (AChE); it is widely used in agriculture and in our study area it is found at concentrations of 0.14–15.24 μg L^{-1} (Cornejo et al., 2019). Chlorothalonil ($C_8Cl_4N_2$) is a polychlorinated aromatic compound found in concentrations of 0.26–0.38 μg L^{-1} in sediments of the study catchment (MIDA, 2016). Both pesticides were lethal for the three most common detritivores in our study area in acute toxicity bioassays (Cornejo et al., 2021), and the fungicide affected AHs, a detritivore caddisfly and decomposition rates in a microcosm experiment conducted in northern Spain (Cornejo et al., 2020a).

The two pesticides were purchased in local agricultural stores authorized for the sale and distribution of agrochemicals, and dilutions were made with filtered water from the Chiriquí Viejo stream collected within the La Amistad International Park (PILA), where no pesticides had been detected (Cornejo et al., 2020b). Given that we could not measure concentrations during the experiment, due to logistical and financial limitations, we used nominal concentrations that were prepared from two stock solutions: a 100 mg L^{-1} stock solution, which was used to prepare the low experimental concentration (0.01 μg L^{-1}), and a 1000 mg L^{-1} stock solution, used for the high experimental concentration (1.0 μg L^{-1}). Solutions were prepared using glass volumetric flasks and plastic micropipette tips, following usual protocols. We acknowledge that the use of nominal concentrations allows our results to be compared to those of other studies but precludes their use in regulatory risk assessment (Von Fumetti and Blaurock, 2018).

2.3. Leaf litter

We used leaf litter of *Alnus acuminata* Kunth. (Betulaceae), an alder species that is common in the riparian vegetation of the Chiriquí Viejo river. This species is often used in decomposition studies (Encalada et al., 2010; Rincón et al., 2017; Cornejo et al., 2020b) because of the high quality of its leaf litter, with specific leaf area of 11.5 ± 1.9 mm^2 mg^{-1} and nitrogen concentration of 2.39 ± 0.08% (mean ± SE; Boyero et al., 2021). We collected recently senesced leaf litter from the riparian forest floor in the study catchment (8.89 °N, 82.62 °W) in September 2019. In the laboratory, leaf litter was air dried; cut in ca. 2 × 2 cm fragments excluding the basal petiole insertion; thoroughly mixed to avoid any potential bias that differences in terrestrial fungal colonization among leaves could introduce; and stored within cardboard boxes until its use.

2.4. Detritivores

We selected three detritivore taxa that are common in the Chiriquí Viejo river upper catchment (Cornejo et al., 2019, 2020b): *Anchytarsus* sp. (Coleoptera: Ptilodactylidae), *Hyalella* sp. (Amphipoda: Hyalellidae) and *Lepidostoma* sp. (Trichoptera: Lepidostomatidae). We collected them in 1st and 2nd order streams located within protected areas (Barú Volcano National Park and PILA), to guarantee that they had not been previously exposed to pesticides (Fig. S1). The average pH was 7.5; water temperature 14.4 °C; conductivity 43.7 μS cm^{-1}; turbidity 0.3 NTU; and dissolved oxygen saturation 97.6%. For detritivore collection we used litter bags filled with *A. acuminata* leaf litter and submerged in the stream for 15 days, or multihabitat sampling using a D-net with 0.5-mm mesh. Detritivores were placed in plastic containers filled with stream water and litter, with constant aeration, and transferred to the experimental facilities where they were counted and identified using a stereoscope. They were acclimatized for 96 h in aquariums with 1–2 L of stream filtered water with constant aeration

(provided through syringe tips connected to a pump) at a temperature of 14.4 ± 0.2 °C and with a light: dark regime of 12:12 h. They were fed with fragments of *A. acuminata* leaf litter for the first 48 h of acclimatization, and then fasted for 24 h before starting the experiment in order to homogenize their initial condition. We used 504 individuals in the experiment (168 individuals per species). Another 90 individuals (30 per species) were used to estimate initial biomass of experimental individuals, based on body length (L, mm)/dry mass (DM; g) relationships. The relationships were: *Anchytarsus*, DM = $0.0004e^{0.1739x}$, r^2 = 0.81; *Hyalella*, DM = $0.0004e^{0.2111x}$, r^2 = 0.65; and *Lepidostoma*, DM = $0.0003e^{0.2624x}$, r^2 = 0.37 (Fig. S2). The estimated mean DM ± SD of experimental individual was: *Anchytarsus*, 15.5 ± 9.2 mg; *Hyalella,* 1.4 ± 0.52 mg; *Lepidostoma*, 1.5 ± 1.03 mg.

2.5. Aquatic hyphomycetes

In November 2019, we collected natural leaves in different stages of decomposition from the benthos of the Chiriquí Viejo stream, a tributary of the main river (8,893°N; 82,619°W). These leaves were incubated at 14.4 ± 0.2 °C for three days in a glass aquarium (2.2 L) with filtered tap water (100 μm), renewed every 24 h. Aliquots of 10 mL of this water were used to inoculate each microcosm at the beginning of the experiment (day 0), and four extra 25-mL aliquots were used to characterize the initial AH assemblage. These samples were pre-stained with 2 drops of trypan blue, preserved with 2 mL of 35% formalin and adjusted to 35 mL with distilled water. In order to ensure a uniform distribution of conidia, each sample was mixed with a magnetic stirrer and received 100 μL of Triton X-100 (0.5%). A sub-sample of 10 mL was filtered (25-mm diameter, pore size 5 μm, Millipore SMWP, Millipore Corporation) with gentle vacuum (Descals, 2005). Immediately after, filters were stained with two drops of 0.4% trypan blue, and conidia were identified and counted at 200 × magnification (Gulis et al., 2005). The AH assemblage in the inoculum was composed of 11 taxa (Table S1), with *Tetrachaetum elegans* Ingold, *Lunulospora curvula* Ingold and *Alatospora acuminata* Ingold being the dominant species.

2.6. Experimental procedure and sample processing

The experiment was carried out at the PILA station, with temperature of 14.4 ± 0.2 °C (which was the temperature in nearby streams at the time of the experiment) and a light:dark regime of 12:12 h, reflecting natural conditions at the time. Microcosms consisted of 500-mL glass jars, which were aerated through a system of syringes connected to hoses that were fed from a pump. Each microcosm contained 300 mL of filtered (100 μm) water from the Chiriquí Viejo stream; 0.700 ± 0.01 g of leaf litter accessible to detritivores; 0.300 ± 0.01 g of leaf litter enclosed within a 100-μm mesh bag, which served to determine microbial decomposition; and three detritivore individuals.

The 168 microcosms were homogeneously distributed among the different treatments of two factors, (1) the pesticide factor and (2) the detritivore factor, with six replicates for each combination of factor and treatment (Fig. S3). The pesticide factor had seven treatments corresponding to different concentrations of pesticides individually and together: control (no pesticides); the insecticide in low concentration (0.01 μg L^{-1}); the insecticide in high concentration (0.1 μg L^{-1}); the fungicide in low concentration (0.01 μg L^{-1}); the fungicide in high concentration (0.1 μg L^{-1}); both pesticides in low concentration (0.01 μg L^{-1}); and both pesticides in high concentration (0.1 μg L^{-1}). The detritivore factor had four treatments: *Anchytarsus*, *Hyalella*, *Lepidostoma*, and the 3-species assemblage.

In each microcosm the litter was immersed in filtered tap water for 48 h, with water replacement at 24 h, to promote the leaching of soluble compounds (Bärlocher et al., 2010). After this time, we replaced the water with fresh filtered tap water and added the microbial inoculum and the different concentrations of the tested pesticides. The experiment ran for 20 days with water/pesticide replacement on days 5, 10 and 15. Each replacement was done by filtering the water through a mesh in which the detritivores were carefully kept, and thoroughly and rapidly washing the microcosm before being refilled and detritivores returned; this minimized the risk of pesticide concentration increasing during the experiment due to its adherence to the microcosm walls, although there may have been some adherence to leaf litter, which was not controlled for.

Detritivore survival was checked every day. At the end of the experiment (day 20), the water was filtered (100 μm) to retain all the litter fragments and the detritivores. The litter accessible to detritivores and that within fine mesh bags was separately oven dried (70 °C, 48 h), weighed to calculate DM, and incinerated (500 °C, 4 h) and reweighed to calculate ash-free dry mass (AFDM). Detritivores were then fasted for 48 h within the microcosms with 100 mL of fresh filtered tap water without litter or pesticides. On day 22, a 25-mL aliquot of water from each microcosm was taken to evaluate HA sporulation rate and assemblage composition following the procedures described in Cornejo et al. (2020a).

2.7. Data analysis

We calculated our response variables as follows. Detritivore survival was the median time to death (MTD; Bland and Altman, 1998); detritivore growth was the proportional change in detritivore DM [(final DM − initial DM)/initial DM], but high mortality precluded use of this variable. AH assemblage structure was explored with non-metric multidimensional scaling (NMDS) based on the Bray-Curtis similarity index of conidial abundance data, using the "metaMDS" function of the *vegan* package of R statistical software (R Core Team, 2019), separately for each detritivore treatment. Differences among treatments (i.e., pesticide concentrations and detritivore diversity levels) were tested with permutational analysis of variance (PERMANOVA, using the "adonis" function, in the *vegan* package). AH sporulation rate was measured as the number of released conidia per mg of leaf litter (AFDM) per day. AH taxon richness was the number of species; total decomposition rate was the proportional leaf litter mass loss [LML = (final AFDM − initial AFDM)/initial AFDM)] for leaf litter accessible to detritivores (with initial AFDM estimated based on the extra microcosms used to quantify leaching losses), divided by detritivore initial DM to avoid the possible effect of interspecific differences; and microbial decomposition rate was LML for leaf litter enclosed within mesh bags.

To test our hypotheses, we used three types of analysis, depending on the response variable. Firstly, detritivore survival was examined with the Kaplan-Meier product-limit method ("surv" function, *survival* package), and differences among treatments were examined with a Cox proportional-hazards model ("cox.ph" function, survival package; e.g., Ferreira et al., 2010). We analysed the whole dataset and then each detritivore treatment separately (*Anchytarsus*, *Hyalella*, *Lepidostoma* and the mixture). Pesticide treatment was included as a single factor that included both the type of pesticide and the concentration, because this model did not allow nested factors (see below). Secondly, effects on AH assemblage structure were examined (separately for each detritivore treatment) using the "adonis" function of the *vegan* package (De Cáceres, 2013). The contribution of each AH species to each treatment was examined with the similarity percentage procedure

(SIMPER; "simper" function, *vegan* package), and an indicator value index ("multipatt" function of the *indicspecies* package) was used to identify the most representative species of each assemblage. Thirdly, effects of pesticides on all other response variables (AH sporulation rate and taxon richness, and total and microbial decomposition) were examined by calculating an effect size for each treatment [effect size = (treatment value − mean control value)/mean control value]. We calculated ordinary nonparametric bootstrapped 95% confidence intervals using the BCa method, based on 999 bootstrap replicates ("boot" function, boot package; Canty and Ripley, 2016; Davison and Hinkley, 1997); intervals containing the zero value indicated no pesticide effect. We then used linear models ("lm" function, nlme package; Pinheiro et al., 2020) to compare effect sizes (our response variables) between pesticide types (insecticide, fungicide or both), pesticide concentrations (high or low, nested within each pesticide type), detritivore diversity levels (1 or 3 species) and detritivore species (*Anchytarsus*, *Hyalella* and *Lepidostoma* nested within the 1-species level); these were all fixed factors in the models, which were followed by multiple comparisons (TukeyHSD function, vegan package).

3. Results

Detritivore survival varied depending on pesticide treatment (Fig. 1, Table S2). The highest survival occurred in the controls with no pesticide (with mortality only of *Hyalella*), and the lowest generally occurred at the high concentration of the insecticide and the combination of both pesticides (Table S3). There was, however, some variation among detritivore species, with the highest survival in *Anchytarsus* and the lowest in *Hyalella* (Table S3). There was an effect of detritivore diversity, but it varied with pesticide treatment, as indicated by a significant interaction between factors in the overall model and for two of the species (Table S2).

Mean sporulation rate observed in control microcosms varied from 4734 to 10415 conidia g^{-1} d^{-1} and mean taxon richness was 2.7–4.3 (Fig. S4, Table S1). Considering all microcosms, we found a total of 15 AH sporulating taxa (Table S1), but the number of sporulating taxa per microcosm was low (2–5). There were 1–2 dominant taxa per microcosm (mostly *Flagellospora fusarioides* and *Tetrachaetum elegans*), with other taxa (up to 7) having minor contribution to abundance. Only two species were indicators of particular treatments; *L. curvula* was associated with low fungicide and high insecticide treatments ($p = 0.005$) and *Articulospora tetracladia* Ingold was associated with high insecticide treatments ($p = 0.010$). Community structure varied significantly depending on pesticide treatment in microcosms with *Anchytarsus*, *Hyalella* and the mixture (Fig. 2; Table S4). The pesticide effect on sporulation rate differed between pesticide types (being greater for the fungicide than for the insecticide or their combination) and increased with pesticide concentration, with no variation associated to the diversity or identity of detritivores (Fig. 3; Table 1, Table S5). The pesticide effect on AH taxon richness was greater for the fungicide than the insecticide (and intermediate for the combination) and again increased with concentration. It was also greater in microcosms with *Hyalella* than in those with *Lepidostoma* (Fig. 3; Table 1, Table S5).

In the absence of pesticides, decomposition of the litter accessible to detritivores ranged from 0.09 to 0.28 proportion of LML (for *Hyalella* and *Anchytarsus*, respectively), with clear influence of detritivore biomass. When decomposition was standardized by biomass (i.e., expressed as prop. LML per initial detritivore mg), microcosms with *Lepidostoma* showed much higher values than the others (Fig. S5). Microbial decomposition showed lower and more consistent values across treatments (0.07–0.08 prop. LML), accounting for an average of 42% of total decomposition (Fig. S5). The pesticide effect on total decomposition was higher for the combination of pesticides than for individual pesticides, increased with concentration, was higher for single-species detritivore treatments

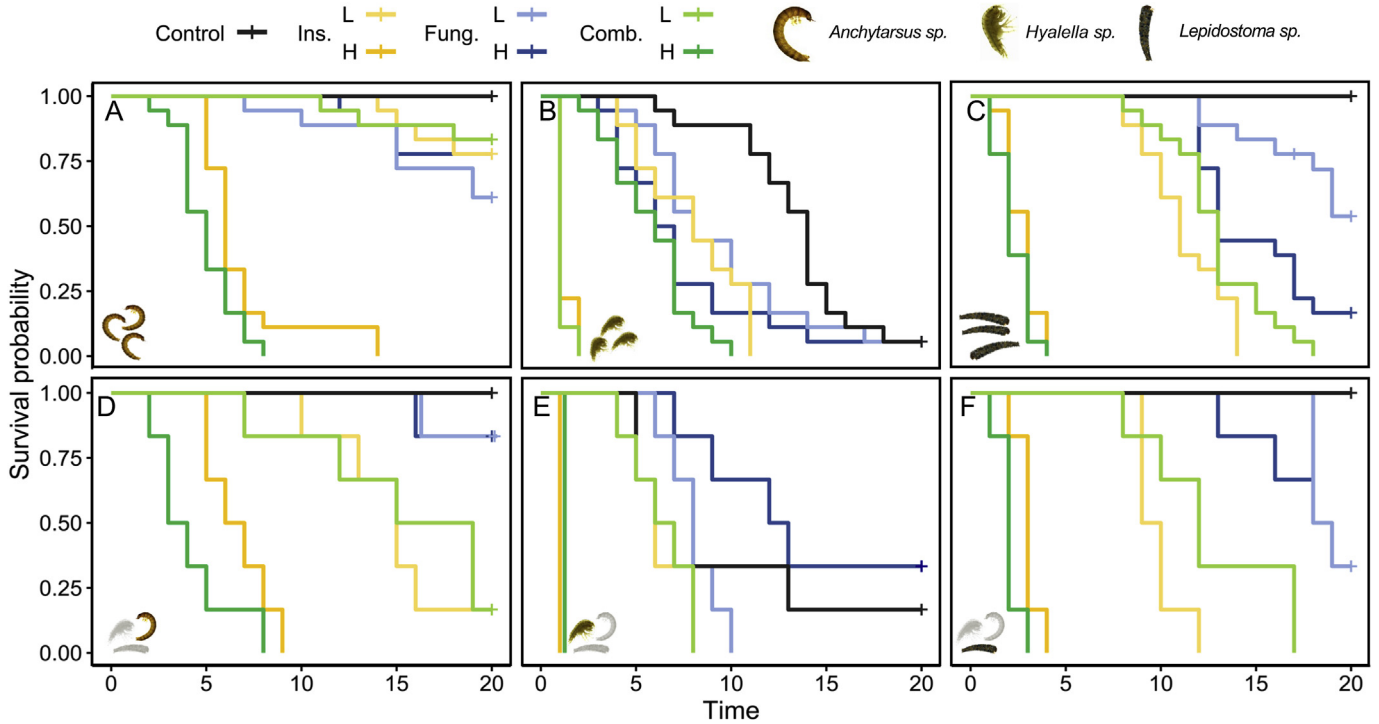

Fig. 1. Detritivore survival probability through experimental time (d) in microcosms with different pesticide types (Ins., chlorpyrifos; Fung., chlorothalonil; Comb., combination of both pesticides) and concentrations (L, low; H, high), and different detritivore diversity levels (1 or 3 species) and species (*Anchytarsus*, *Hyalella* or *Lepidostoma*).

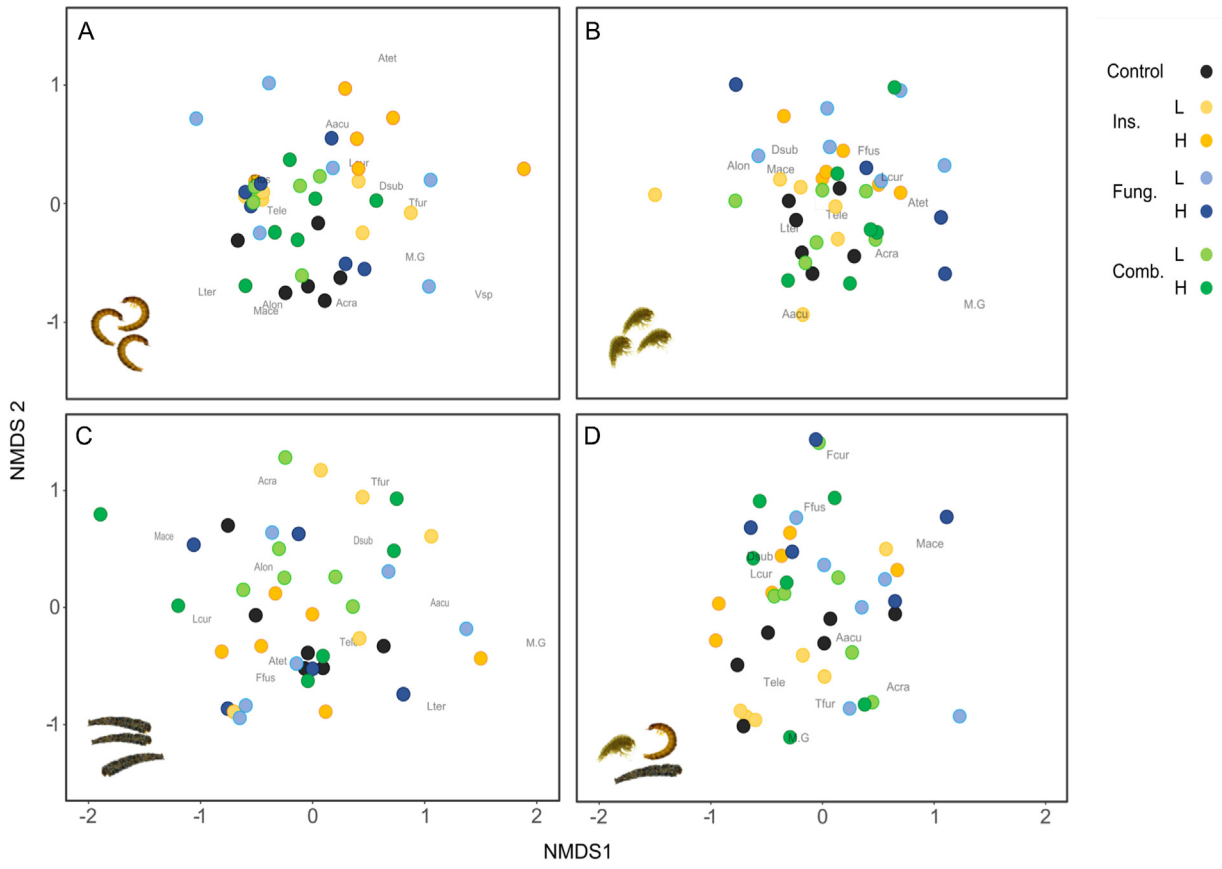

Fig. 2. Non-metric multidimensional scaling (NMDS) ordination of AH assemblages for microcosms with different pesticide types and concentrations and different detritivore diversity levels and species (see Fig. 1 legend). Aac, *Alatospora acuminata*; Acra, *Angillospora crassa*; Alon, *Angillospora longissima*; Atet, *Articulospora tetracladia*; Dsub, *Dactylella submerse*; Fcur, *Flagellospora curvula*; Ffus, *Flagellospora fusarioides*; Lter, *Lemonniera terrestris*; Lcur, *Lunulospora curvula*; Mace, *Mycocentrospora acerina*; Tele, *Tetrachaetum elegans*; Tfur, *Tetracladium furcatum*; Tace, *Trisulcosporium acerinum*; Vsp, *Variocladium* sp.

than for the mixture, and was higher for microcosms with *Hyalella* or *Lepidostoma* than for those with *Anchytarsus* (Fig. 4; Table 1, Table S6). The pesticide effect on microbial decomposition was higher for the fungicide and the combination of pesticides than for the insecticide, and did not vary with other treatments.

4. Discussion

Our results reveal detrimental effects of two pesticides, commonly used in agriculture in our study area, on stream detritivores, aquatic hyphomycetes and a key ecosystem process. Pesticides are known to contaminate tropical streams and, together with associated stressors such as organic pollution and habitat loss, alter their assemblages (Fugère et al., 2016; Rasmussen et al., 2016; Cornejo et al., 2019) and impair their functioning (Jinggut et al., 2012; Dawoud et al., 2017; Cornejo et al., 2020b; Fugère et al., 2020). However, experimental evidence on the mechanisms driving these effects in the tropics is virtually lacking. In temperate areas, multiple laboratory experiments have demonstrated that pesticides can reduce the survival and fitness of stream detritivores (Flores et al., 2014; Zubrod et al., 2014; Cornejo et al., 2020a); alter the composition of AH assemblages and reduce their biomass, sporulation rate and taxon richness (Zubrod et al., 2015, 2017); and reduce decomposition rates (Rasmussen et al., 2012a; Cornejo et al., 2020a). Exploring these causal relationships in tropical areas is important because agriculture is expanding there at alarming rates (Gibbs et al., 2010), with pesticides often used at higher concentrations than elsewhere because of less restrictive regulations (Castillo et al., 1997), poorer management practices and lower awareness of farmers and the community, as well as the warmer and wetter climates that promote pesticide runoff and foster the development of pest outbreaks (Lewis et al., 2016).

4.1. A common-use insecticide reduced the survival of tropical detritivores

We found that chlorpyrifos, an insecticide commonly used in our study area and in other tropical areas (Bajet et al., 2012; Diepens et al., 2014; Sumon et al., 2016), caused elevated mortality in three species of common detritivores that belonged to different orders. This agrees with results of an acute toxicity test finding very high sensitivity of these detritivores to chlorpyrifos (Cornejo et al., 2021) and with previous work with *Hyalella azteca* (Phipps et al., 1995; Trimble and Lydy, 2006), sugesting that our exposure concentrations were close to the nominal values. Here, individuals started to die immediately upon exposure, with all individuals being dead at day 1 (*Hyalella*) to 15 (*Anchytarsus*) at the higher concentration of the insecticide, applied alone or in combination with the fungicide chlorothalonil. The fungicide itself also caused detritivore mortality, as previously shown in the above-mentioned experiment (Cornejo et al., 2021) and also for a temperate caddisfly (Cornejo et al., 2020a), although rates were lower than for the insecticide. The fact that individuals were fasted before the experiment could have intensified the effects of pesticides, but we note that we used concentrations well below those reported in our study area (0.14–15.24 μg L^{-1} for chlorpyrifos and 0.26–0.38 μg L^{-1} for

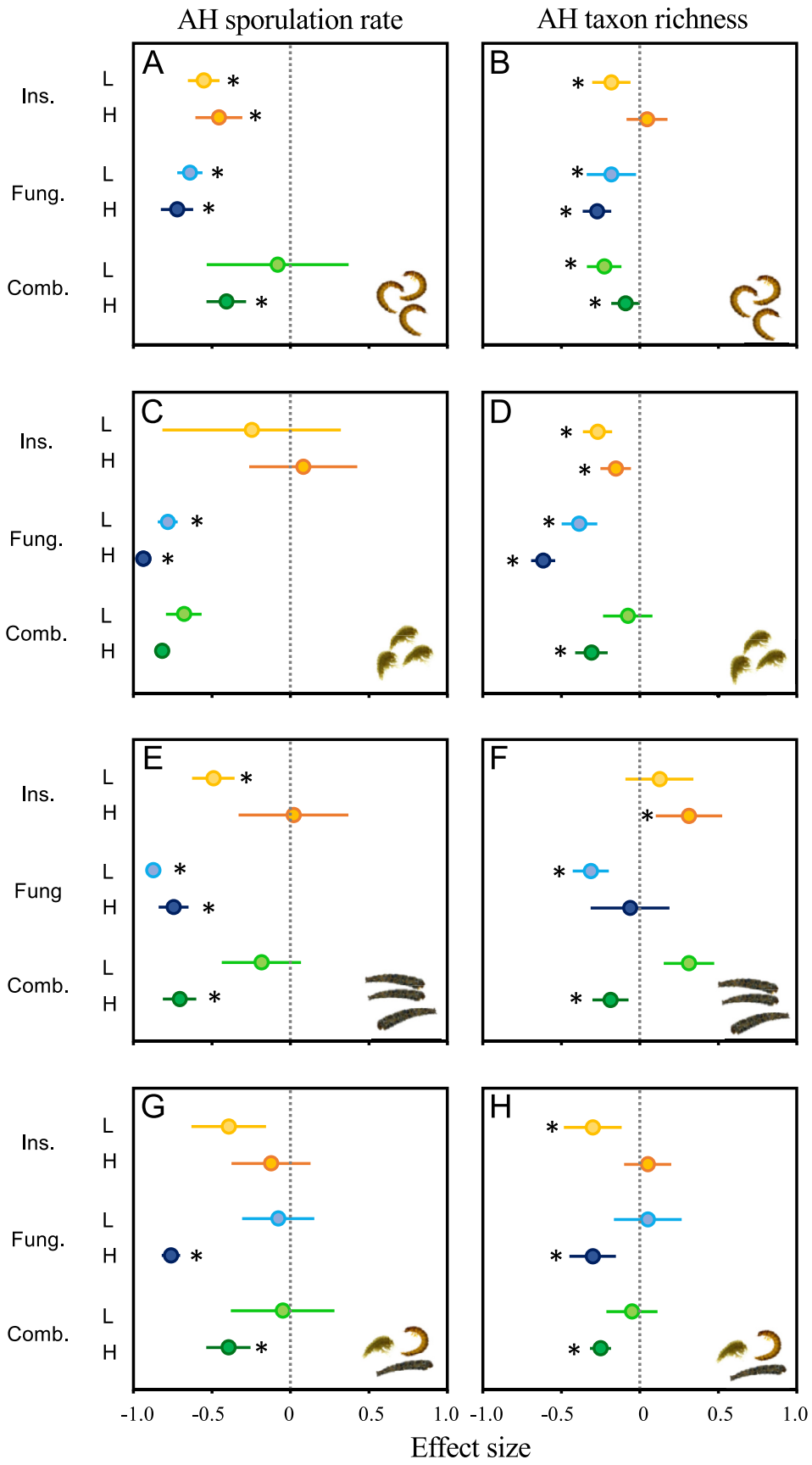

Fig. 3. Pesticide effect size [(treatment value − mean control value)/mean control value] on aquatic hyphomycete sporulation rate and taxon richness (mean ± SE, n = 6), for microcosms with different pesticide types and concentrations and different detritivore diversity levels and species (see Fig. 1 legend).

Table 1
Linear models testing the influence of pesticide type (Ins., chlorpyrifos; Fung., chlorothalonil or Comb., combination of pesticides), pesticide concentration (low or high; nested within pesticide type), detritivore diversity (1 or 3 species) and detritivore species (*Anchytarsus*, *Hyalella* or *Lepidostoma*; nested within detritivore diversity) on four response variables, all quantified as a pesticide effect size [(treatment value − mean control value)/mean control value] on a given parameter (aquatic hyphomycete sporulation rate and taxon richness, and total and microbial decomposition). Interactions were allowed but results were nonsignificant, so we present models without interactions. We show the degrees of freedom (df), *F*-statistic, *P*-value and results of post-hoc multiple comparisons for significant effects. *Anc, Anchytarsus; Hya, Hyalella; Lep, Lepidostoma.*

Response variable	Factor/interaction	df	*F*	*P*	Post-hoc comparisons
AH sporulation rate	Pest. type	2	6.99	0.001	Fung. > Ins., Comb.
	Pest. concentration	3	3.04	0.031	High > Low
	Detr. diversity	1	3.84	0.052	–
	Detr. identity	2	0.24	0.791	–
AH taxon richness	Pest. type	2	4.41	0.014	Fung. > Ins.
	Pest. concentration	3	3.03	0.032	High > Low
	Detr. diversity	1	0.01	0.918	–
	Detr. identity	2	7.64	<0.001	*Hya* > *Lep*
Total decomposition	Pest. type	2	13.90	<0.001	Comb. > Fung., Ins.
	Pest. concentration	3	31.82	<0.001	High > Low
	Detr. diversity	1	12.48	<0.001	1 sp. > 3 spp.
	Detr. identity	2	9.23	<0.001	*Hya*, *Lep* > *Anc*
Microbial decomposition	Pest. type	2	66.34	<0.001	Fung., Comb. > Ins.
	Pest. concentration	3	1.83	0.145	–
	Detr. diversity	1	0.36	0.549	–
	Detr. identity	2	1.50	0.227	–

chlorothalonil; Cornejo et al., 2019), which suggests that effects in the field may be even greater than those revealed in our experiment.

Some experimental studies conducted in temperate areas have also reported lethal effects of exposure to pesticides individually or in combination but, in contrast to our findings, effects of fungicides were greater than those of insecticides. For example, Dawoud et al. (2017) reported increased mortality of the isopod *Asellus aquaticus* exposed to the fungicide azoxystrobin alone and in combination with insecticide lindane, with no mortality from lindane alone; and Flores et al. (2014) reported greater mortality of the amphipod *Echinogammarus berilloni* exposed to the fungicide imazalil compared to the insecticide diazinon, with effects of their combination being intermediate. These differences could be due to the fact that these two studies used crustaceans, which are not target organisms of insecticides and are sensitive to fungicides (Zubrod et al., 2014, 2017), whereas we used two insects and one crustacean, *Hyalella*, which did show greater mortality than the two insects when exposed to the fungicide.

Differences in the degree of sensitivity of different species to the pesticides were expected. Available evidence suggests that sensitivity is determined by species-specific physiological and morphological traits, as well as the toxicodynamics of the chemical (Van den Berg et al., 2019). For example, body size may play a role (Tonin et al., 2018). Thus, *Hyalella*, the most sensitive species in our experiment, was also the smallest and the least sensitive, *Anchytarsus* was the largest. Differences between *Hyalella* and the other species could also be related to traits specific to crustaceans, either anatomical (e.g., the lower complexity of their digestive tract or their different respiratory structures; Wogram and Liess, 2001) or physiological (e.g., their energy investment in reproduction as adults, in contrast to insect larvae; Castiglioni et al., 2018), but differences between the two insects are more likely related to body size. It is also possible that survival of *Hyalella* was reduced by the absence of mineral substrate in the microcosms, as crustaceans often require higher mineral concentrations than insects for their development (Wogram and Liess, 2001).

Interestingly, we observed that, in some cases, the survival of a given species depended on whether it shared the microcosm with the other species. This mostly occurred for *Anchytarsus*, which survived longer on its own than in the presence of *Hyalella* and *Lepidostoma* at the high concentrations of the insecticide and the combination of both pesticides. This could be due to a release from interspecific competition for resources such as food and space (Bastian et al., 2008; Rasmussen et al., 2012a), as resource limitation can accentuate the effects of pesticides (Beketov and Liess, 2005).

4.2. Pesticides altered microbial decomposer assemblages and their performance

AH taxon richness in our study was low overall, with 15 taxa in total, including 10 taxa previously reported (Bärlocher et al., 2010; Cáceres et al., 2015) and 2 unidentified. The dominant species during the experiment were *F. fusarioides* and *T. elegans*, of which only the latter was dominant in the inoculum. This cosmopolitan species is commonly reported on litter decomposition studies (Flores et al., 2014; Fernández et al., 2016; Biasi et al., 2017; Schreiner et al., 2018), has large conidia and is tolerant to organic pollutants (e.g., Pérez et al., 2013). Its abundance declined when it was exposed to the insecticide and the fungicide. Other species (*F. fusarioides, Articulospora tetracladia* Ingold, and *L. curvula*) increased their sporulation when exposed to the insecticide but reduced it when exposed to the fungicide. Our analysis indicated that the latter two were tolerant of pesticides. *Articulospora tetracladia* produce medium-size tetraradiated conidia capable of secondary conidiation, which might increase their frequency in conidial assemblages (Pérez et al., 2012). Similarly, species with small and simple-shaped conidia, such as the other two dominant taxa, usually are fast colonizers of decomposing litter and dominate sporulation peaks (Treton et al., 2004). Previous studies have reported contrasting results for *L. curvula,* usually associated with warmer and nutrient-rich conditions (Pérez et al., 2018), which is affected both negatively (Duarte et al., 2008; Flores et al., 2014) and positively (Fernandez et al., 2015; Cornejo et al., 2020a) by fungicides and other contaminants. Similarly, responses of *A. tetracladia* to pesticides are variable (Pérez et al., 2018; Schreiner et al., 2018). These contradictory results highlight the need for more studies evaluating the tolerance of AHs to chemical stressors, particularly

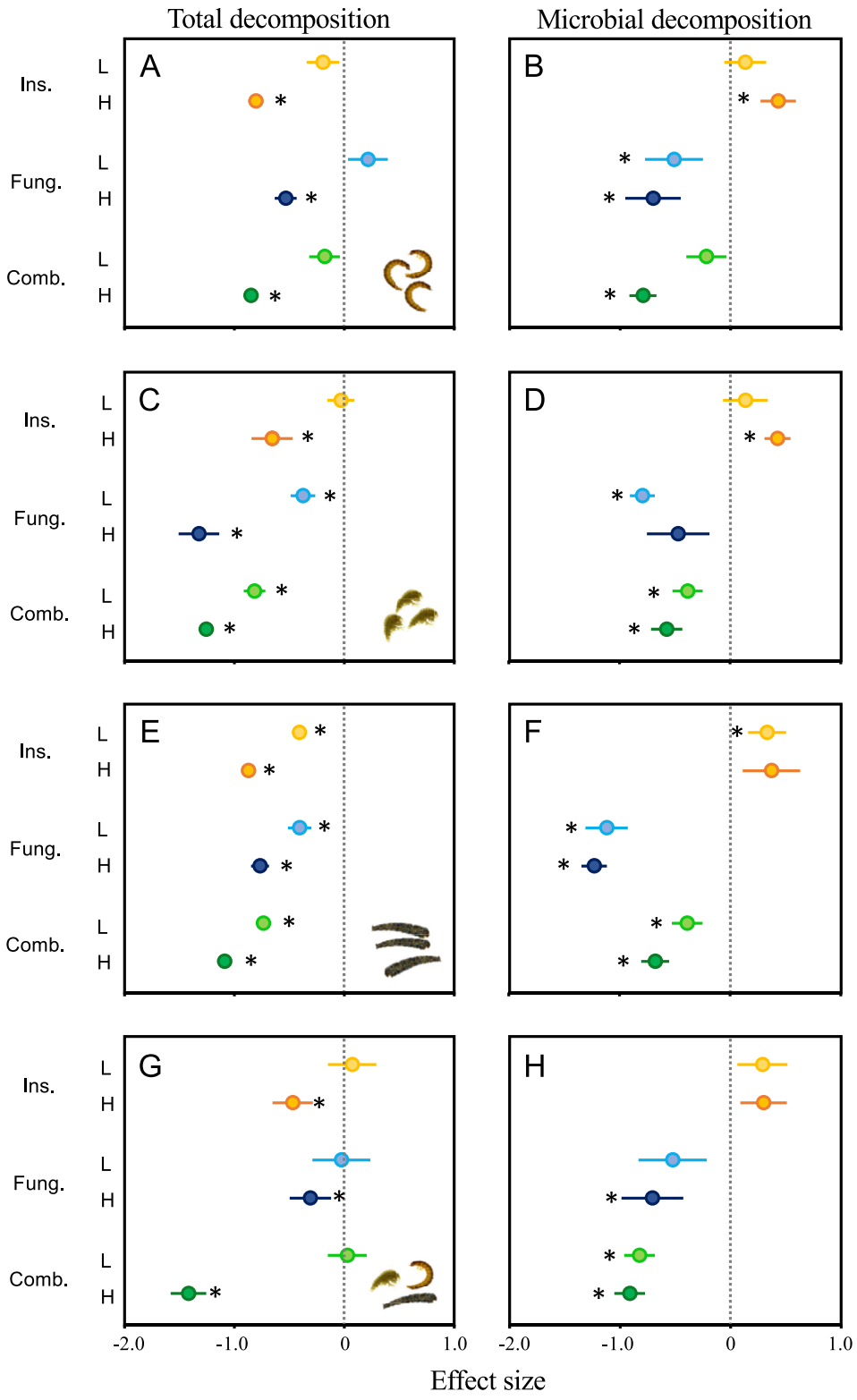

Fig. 4. Pesticide effect size [(treatment value − mean control value)/mean control value] on total and microbial decomposition (proportion of litter mass loss, mean ± SE, n = 6) for microcosms with different pesticide types and concentrations and different detritivore diversity levels and species (see Fig. 1 legend).

in tropical regions.

We found that both the fungicide and the insecticide caused changes in AH assemblage structure, sporulation rate and taxon richness, but only effects of the fungicide were negative (i.e., reduced sporulation rate and taxon richness) and depended on concentration. This was expected given that fungi are the main target of fungicides (Flores et al., 2014; Zubrod et al., 2015; Cornejo et al., 2020a). Interestingly, we observed higher sporulation rate and taxon richness in microcosms with the insecticide, suggesting a subsidy effect *sensu* Odum et al. (1979), as previously observed for

the insecticide lindane (Dawoud et al., 2017) and other toxic compounds (Swift et al., 1988; Cornejo et al., 2020a). Changes also depended on the diversity and identity of the detritivores present in microcosms, with the lowest sporulation rates and taxon richness in microcosms containing *Anchytarsus* or the mixture, and the highest values in microcosms with *Hyalella*.

The combination of pesticides had the greatest effects on decomposition but detritivore diversity attenuated these effects.

As expected, following studies conducted in temperate areas (Cornejo et al., 2020a), exposure to pesticides significantly reduced total decomposition, and this effect increased with concentration. Importantly, total decomposition was more strongly reduced in microcosms containing both pesticides than in those containing a single pesticide, suggesting that they operated differently and their effects were additive (Jackson et al., 2016). For microbial decomposition, differences occurred between the insecticide (which actually enhanced decomposition) and the fungicide or their combination. Others have found increased microbial decomposition in the presence of insecticides (Dimitrov et al., 2014; Zubrod et al., 2014, 2017), or that the presence of an insecticide moderated the reduction in microbial decomposition caused by a fungicide (Rasmussen et al., 2012b).

We found that pesticide effects on total decomposition were greater for single-species detritivore treatments than for the 3-species mixture. The fact that this did not occur for microbial decomposition indicates that effects were due to detritivores. There are two plausible explanations for this pattern: firstly, the negative effects of pesticides on litter processing by one species could be mitigated by the presence of others, possibly through facilitation (Tonin et al., 2018); and secondly, litter processing by the species that is less affected by pesticides (*Anchytarsus* in this case) compensates for that of the more affected species (i.e., compensatory species dynamics; Vinebrooke et al., 2004). Our experiment does not allow us to identify which of these mechanisms might underlie the buffering of pesticide effects provided by detritivore diversity, but it suggests that loss of detritivore species might exacerbate the effects of pesticides on stream ecosystem functioning.

5. Conclusion

Our microcosm experiment demonstrated detrimental effects of commonly used pesticides (applied at concentrations well below those reported in our study area) on tropical detritivores and aquatic hyphomycetes, which translated into impaired ecosystem functioning. Thus, our results support those of field studies that revealed impacts of agricultural practices in tropical streams (Lewis et al., 2016; Rasmussen et al., 2016; Cornejo et al., 2020b). Clearly, there are factors that operate in streams that cannot be considered in a microcosm experiment (e.g., stream flow and substrate) and could interact with the results found here. However, by using microcosms we were able to isolate the effect of pesticides from those of other factors that cannot be separated in the field (e.g., eutrophication and habitat loss). Importantly, we underscore a key role of detritivore diversity in the maintenance of ecosystem functioning when streams are exposed to pesticides, which is possibly driven by facilitation or compensatory species dynamics. We thus highlight the importance of considering detritivore diversity loss as one of the multiple stressors associated with agricultural activities, especially in tropical regions, where their assemblages already are mostly less diverse than in temperate areas (Boyero et al., 2012).

Credit author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

ANAM, C.A.T.I.E., 2014. Plan de Manejo de la Cuenca del Río Chiriquí Viejo.
Bajet, C.M., Kumarb, A., Calingaciona, M.N., Narvacan, T.C., 2012. Toxicological assessment of pesticides used in the Pagsanjan-Lumban catchment to selected non-target aquatic organisms in Laguna Lake, Philippines. Agric. Water Manag. 106, 42–49.
Bärlocher, F., Helson, J.E., Williams, D.D., 2010. Aquatic hyphomycete communities across a land-use gradient of Panamanian streams. Fundamental and Applied Limnology/Archiv für Hydrobiologie 177, 209–221.
Bastian, M., Pearson, R.G., Boyero, L., 2008. Effects of diversity loss on ecosystem function across trophic levels and ecosystems: a test in a detritus-based tropical food web. Austral Ecol. 33, 301–306.
Beketov, M.A., Liess, M., 2005. Acute contamination with esfenvalerate and food limitation: chronic effects on the mayfly, Cloeon dipterum. Environ. Toxicol. Chem. 24, 1281–1286.
Biasi, C., Graçaa, M.A.S., Santos, S., Ferreira, V., 2017. Nutrient enrichment in water more than in leaves affects aquatic microbial litter processing. Oecologia 184, 555–568.
Bland, J.M., Altman, D.G., 1998. Survival probabilities (the Kaplan-Meier method. BMJ 317, 1572–1580.

Boyero, Luz, Pérez J, López-Rojo N, Tonin AM, Correa-Araneda F, Pearson RG, Bosch J, Albariño RJ, Anbalagan S, Barmuta LA, Beesley L, Bunn SE, Burdon FJ, Caliman A, Callisto M, Campbell IC, Cardinale BJ, Casas JJ, Chará-Serna AM, Ciapała S, Chauvet E, Colón-Gaud C, Cornejo A, Davis AM, Degebrodt M, Dias ES, Díaz ME, Douglas MM, Elosegi A, Encalada AC, E. de Eyto, Figueroa R, Flecker AS, Fleituch T, Frainer A, França JS, García EA, García G, García P, Gessner MO, Giller PS, Gómez JE, Gómez S, Gonçalves JFJr., Graça MAS, Hall ROJr., Hamada N, Hepp LU, Hui C, Imazawa D., Iwata T, Edison S. A. Jr, Kariuki S, Landeira-Dabarca A, Leal M, Lehosmaa K, M'Erimba C, Marchant R, Martins RT, Masese F, Maul M, McKie BG, A. Medeiros O, Middleton JA, Muotka T, Negishi JN, Pozo J, Ramírez A, Rezende RS, Richardson JS, Rincón J, Rubio-Ríos J, Serrano C, Shaffer AR, Sheldon F, Swan CM, Tenkiano NSD, Tiegs SD, Tolod JR, Watson A, Yegon MJ, Yule CM., 2021. Latitude dictates plant diversity effects on instream decomposition. Sci. Adv. 7 (eabe7860). https://advances.sciencemag.org/content/7/13/eabe7860.

Boyero, L., Cardinale, B.J., Bastian, M., Pearson, R.G., 2014. Biotic vs. abiotic control of decomposition: a comparison of the effects of simulated extinctions and changes in temperature. PloS One 9, e87426.

Boyero, L., Pearson, R.G., Dudgeon, D., Ferreira, V., Graça, M.A.S., Gessner, M.O., A J, B., Chauvet, E., Yule, C.M., Albariño, R.J., Ramírez, A., Helson, J.E., Callisto, M., Arunachalam, M., Chará, J., Figueroa, R., J M, M., J F, G., Moretti, M., Chará, A.M., Davie, J.N., Encalada, A., Lamothe, S., Buria, L.M., Castela, J., Cornejo, A., Li, A.O.Y., M'Erimba, C., Villanueva, V.D., Zúñiga, M.C., Swan, C., Barmuta, L.A., 2012. Global patterns of stream detritivore distribution: implications for biodiversity loss in changing climates. Global Ecol. Biogeogr. 21, 134–141.

Boyero, L., Pearson, R.G., Dudgeon, D., Graça, M.A.S., Gessner, M.O., Albariño, R.J., Ferreira, V., Yule, C.M., Boulton, A.J., Arunachalam, M., Callisto, M., Chauvet, E., Ramírez, A., Chará, J., Moretti, M.S., Gonçalves, J.F., Helson, J.E., Chará-Serna, A.M., Encalada, A.C., Davies, J.N., Lamothe, S., Cornejo, A., Li, A.O.Y., Buria, L.M., Villanueva, V.D., Zúñiga, M.C., Pringle, C.M., 2011a. Global distribution of a key trophic guild contrasts with common latitudinal diversity patterns. Ecology 92, 1839–1848.

Boyero, L., Pearson, R.G., Gessner, M.O., Barmuta, L.A., Ferreira, V., Graça, M.A.S., Dudgeon, D., Boulton, A.J., Callisto, M., Chauvet, E., Helson, J.E., Bruder, A., Albariño, R.J., Yule, C.M., Arunachalam, M., Davies, J.N., Figueroa, R., Flecker, A.S., Ramírez, A., Death, R.G., Iwata, T., Mathooko, J.M., Mathuriau, C., Gonçalves, J.F., Moretti, M., Jinggut, T., Lamothe, S., M'erimba, C., Ratnarajah, L., Schindler, M.H., Castela, J., Buria, L.M., Cornejo, A., Villanueva, V.D., West, D.C., 2011b. A global experiment suggests climate warming will not accelerate litter decomposition in streams but may reduce carbon sequestration. Ecol. Lett. 14, 289–294.

Boyero, L., Pearson, R.G., Swan, C.M., Hui, C., Albariño, R.J., Arunachalam, M., Callisto, M., Chará, J., Chará-Serna, A.M., Chauvet, E., Cornejo, A., Dudgeon, D., Encalada, A.C., Ferreira, V., Gessner, M.O., Gonçalves, J.F., Graça, M.A.S., Helson, J.E., Mathooko, J.M., McKie, B.G., Moretti, M.S., Yule, C.M., 2015. Latitudinal gradient of nestedness and its potential drivers in stream detritivores. Ecography 38.

Burdon, F.J., McIntosh, A., Harding, J.S., 2013. Habitat loss drives threshold response of benthic invertebrate communities to deposited sediment in agricultural streams. Ecol. Appl. 23, 1036–1047.

Cáceres, O., Castillo, S., Hofmann, T., 2015. Hifomicetos acuáticos asociados a hojarasca sumergida en el río Majagua, Chiriquí, Panamá. Rev. Mex. Micol. 42, 65–69.

Canty, A., Ripley, B., 2016. Boot: bootstrap R (S-plus) functions. R Foundation for Statistical Computing, Vienna. R package version 1.3–18.

Castiglioni, D.d.S., Streck, M.T., Rodrigues, S.G., Bueno, A.A.d.P., 2018. Reproductive strategies of a population of a freshwater amphipod (Crustacea, Amhipoda, Hyalellidae) from southern Brazil. Biota Neotropica 18, e20170470.

Castillo, L.E., de la Cruz, E., Ruepert, C., 1997. Ecotoxicology and pesticides in tropical aquatic ecosystems of Central America. Environ. Toxicol. Chem. 16, 41–51.

Chauvet, E., Ferreira, V., Giller, P.S., McKie, B.G., Tiegs, S.D., Woodward, G., Elosegi, A., Dobson, M., Fleituch, T., Graça, M.A.S., Gulis, V., Hladyz, S., Lacoursière, J.O., Lecerf, A., Pozo, J., Preda, E., Riipinen, M., Rîşnoveanu, G., Vadineanu, A., Vought, L.B.M., Gessner, M.O., 2016. Litter decomposition as an indicator of stream ecosystem functioning at local-to-continental scales. Large-Scale Ecology: Model Systems to Global Perspectives 99–182.

Cornejo, A., Encina-Montoya, F., Correa-Araneda, F., Rovira, D., García, G., Nieto, G.C.A., Villarreal, V., Jaramillo, N., Pérez, E., Valderrama, A., Pérez, J., Boyero, L., 2021. High sensitivity of invertebrate detritivores from tropical streams to different pesticides. Ecotoxicol. Environ. Saf. 216, 112226.

Cornejo, A., Perez, J., Alonso, A., Lopez-Rojo, N., Monroy, S., Boyero, L., 2020a. A common fungicide impairs stream ecosystem functioning through effects on aquatic hyphomycetes and detritivorous caddisflies. J. Environ. Manag. 263, 110425.

Cornejo, A., Perez, J., Lopez-Rojo, N., Tonin, A.M., Rovira, D., Checa, B., Jaramillo, N., Correa, K., Villarreal, A., Villarreal, V., Garcia, G., Perez, E., Rios Gonzalez, T.A., Aguirre, Y., Correa-Araneda, F., Boyero, L., 2020b. Agriculture impairs stream ecosystem functioning in a tropical catchment. Sci. Total Environ. 745, 140950.

Cornejo, A., Tonin, A.M., Checa, B., Tuñon, A.R., Pérez, D., Coronado, E., González, S., Ríos, T., Macchi, P., Correa-Araneda, F., Boyero, L., 2019. Effects of multiple stressors associated with agriculture on stream macroinvertebrate communities in a tropical catchment. PLoS ONE 14, e0220528.

Davison, A.C., Hinkley, D.V., 1997. Bootstrap Methods and Their Application. Cambridge University Press, Cambridge.

Dawoud, M., Bundschuh, M., Goedkoop, W., McKie, B.G., 2017. Interactive effects of an insecticide and a fungicide on different organism groups and ecosystem functioning in a stream detrital food web. Aquat. Toxicol. 186, 215–221.

De Cáceres, M., 2013. How to use the indicspecies package (ver. 1.7.1). R Project 29.

Descals, E., 2005. Techniques for handling ingoldian fungi. In: Graça, M.A.S., Bärlocher, F., Gessner, M.O. (Eds.), Methods to Study Litter Decomposition: a Practical Guide. Springer, Dordrecht.

Diepens, N.J., Pfennig, S., P J, v d B, Gunnarsson, J.S., Ruepert, C., Castillo, L., 2014. Effect of pesticides used in banana and pineapple plantations on aquatic ecosystems in Costa Rica. J. Environ. Biol. 35, 73–84.

Dimitrov, M.R., Kosol, S., Smidt, H., Buijse, L., Van den Brink, P.J., Van Wijngaarden, R.P., Brock, T.C., Maltby, L., 2014. Assessing effects of the fungicide tebuconazole to heterotrophic microbes in aquatic microcosms. Sci. Total Environ. 490, 1002–1011.

Duarte, S., Pascoal, C., Cassio, F., 2008. High diversity of fungi may mitigate the impact of pollution on plant litter decomposition in streams. Microb. Ecol. 56, 688–695.

Encalada, A.C., Calles, J., Ferreira, V., Canhoto, C.M., GraÇA, M A S, 2010. Riparian land use and the relationship between the benthos and litter decomposition in tropical montane streams. Freshwater Biology.

ETESA, 2008. Resumen Técnico Análisis Regional de Crecidas Máximas de Panamá, Periodo 1971-2006, Panamá, República de Panamá.

Fernández, D., Tummala, M., Schreiner, V.C., Duarte, S., Pascoal, C., Winkelmann, C., Mewes, D., Muñoz, K., Schäfer, R.B., 2016. Does nutrient enrichment compensate fungicide effects on litter decomposition and decomposer communities in streams? Aquat. Toxicol. 174, 169–178.

Fernandez, D., Voss, K., Bundschuh, M., Zubrod, J.P., Schafer, R.B., 2015. Effects of fungicides on decomposer communities and litter decomposition in vineyard streams. Sci. Total Environ. 533, 40–48.

Ferreira, V., GonÇAlves, A.N.A.L., Godbold, D.L., Canhoto, C., 2010. Effect of increased atmospheric CO2 on the performance of an aquatic detritivore through changes in water temperature and litter quality. Global Change Biol. 16, 3284–3296.

Flores, L., Banjac, Z., Farre, M., Larranaga, A., Mas-Marti, E., Munoz, I., Barcelo, D., Elosegi, A., 2014. Effects of a fungicide (imazalil) and an insecticide (diazinon) on stream fungi and invertebrates associated with litter breakdown. Sci. Total Environ. 476–477, 532–541.

Fugère, V., Kasangaki, A., Chapman, L.J., 2016. Land use changes in an afrotropical biodiversity hotspot affect stream alpha and beta diversity. Ecosphere 7, e01355.

Fugère, V., Lostchuck, E., Chapman, L.J., 2020. Litter decomposition in Afrotropical streams: effects of land use, home-field advantage, and terrestrial herbivory. Freshw. Sci. 000–000.

Gessner, M.O., Chauvet, E., 2002. A case for using litter breakdown to assess functional stream integrity. Ecol. Appl. 12, 498–510.

Gessner, M.O., Swan, C.M., Dang, C.K., McKie, B.G., Bardgett, R.D., Wall, D.H., Hättenschwiler, S., 2010. Diversity meets decomposition. Trends Ecol. Evol. 25, 372–380.

Gibbs, H.K., Ruesch, A.S., Achard, F., Clayton, M.K., Holmgren, P., Ramankutty, N., Foley, J.A., 2010. Tropical forests were the primary sources of new agricultural land in the 1980s and 1990s. Proc. Natl. Acad. Sci. U.S.A. 107, 16732–16737.

Graeber, D., Boechat, I.G., Encina-Montoya, F., Esse, C., Gelbrecht, J., Goyenola, G., Gucker, B., Heinz, M., Kronvang, B., Meerhoff, M., Nimptsch, J., Pusch, M.T., Silva, R.C., von Schiller, D., Zwirnmann, E., 2015. Global effects of agriculture on fluvial dissolved organic matter. Scientic Reports 5, 16328.

Gulis, V., Marvanová, L., Descals, E., 2005. An illustrated key to the common temperate species of aquatic hyphomycetes. In: Graça, M.A.S., Bärlocher, F., Gessner, M.O. (Eds.), Methods to Stuty Litter Decomposition A Practcal Guide. Springer, Dordrecht, the Netherlands.

Handa, I.T., Aerts, R., Berendse, F., Berg, M.P., Bruder, A., Butenschoen, O., Chauvet, E., Gessner, M.O., Jabiol, J., Makkonen, M., McKie, B.G., Malmqvist, B., Peeters, E.T., Scheu, S., Schmid, B., van Ruijven, J., Vos, V.C., Hättenschwiler, S., 2014. Consequences of biodiversity loss for litter decomposition across biomes. Nature 509, 218–221.

Ippolito, A., Todeschini, R., Vighi, M., 2012. Sensitivity assessment of freshwater macroinvertebrates to pesticides using biological traits. Ecotoxicology 21, 336–352.

Jackson, M.C., Lowewen, C.J.G., Vinebrooke, R.D., Chimimba, C.T., 2016. Net effects of multiple stressors in freshwater ecosystems: a meta-analysis. Global Change Biol. 22, 180–189.

Jinggut, T., Yule, C.M., Boyero, L., 2012. Stream ecosystem integrity is impaired by logging and shifting agriculture in a global megadiversity center (Sarawak, Borneo). Sci. Total Environ. 437, 83–90.

Lewis, S.E., Silburn, D.M., Kookana, R.S., Shaw, M., 2016. Pesticide behavior, fate, and effects in the tropics: an overview of the current state of knowledge. J. Agric. Food Chem. 64, 3917–3924.

López-Rojo, N., Pérez, J., Pozo, J., Basaguren, A., Apodaka-Etxebarria, U., Correa-Araneda, F., Boyero, L., 2020. Shifts in key leaf litter traits can predict effects of plant diversity loss on decomposition in streams. Ecosystems.

Maltby, L., 1999. Studying stress: the importance of organism-level responses. Ecol. Appl. 9, 431–440.

MIDA, M.d.D.A., 2016. Enfoque integral de contaminantes químicos y biomonitoreo en la cuenca alta del río Chiriquí Viejo. Resultados Preliminares, Proyecto ARCAL RLA 7019 Desarrollo de indicadores para determinar el efecto de plaguicidas, metales pesados y contaminantes emergentes en ecosistemas acuáticos importantes para la agricultura y agroindustria. (Panamá, República de Panamá).

Odum, E.P., Finn, J.T., Franz, E.H., 1979. Perturbation theory and the subsity-stress gradient. Bioscience 349–352.

Pérez, J., Basaguren, A., Descals, E., Larrañaga, A., Pozo, J., 2013. Leaf-litter processing in headwater streams of northern Iberian Peninsula: moderate levels of eutrophication do not explain breakdown rates. Hydrobiologia 718, 41–57.
Pérez, J., Descals, E., Pozo, J., 2012. Aquatic hyphomycete communities associated with decomposing alder leaf litter in reference headwater streams of the Basque Country (northern Spain). Microb. Ecol. 64, 279–290.
Pérez, J., Martínez, A., Descals, E., Pozo, J., 2018. Responses of aquatic hyphomycetes to temperature and nutrient availability: a cross-transplantation experiment. Microb. Ecol. 76, 328–339.
Petchey, O.L., Gaston, K.J., 2006. Functional diversity: back to basics and looking forward. Ecol. Lett. 9, 741–758.
Phipps, G.L., Mattson, V.R., Ankley, G.T., 1995. Relative sensitivity of three freshwater benthic macroinvertebrates to ten contaminants. Environmental Contamination and Toxicology 28, 281–286.
Pinheiro, J.C., Bates, D.M., DebRoy, S., Sarkar, D., Team, R.C., 2020. Nlme: linear and nonlinear mixed effects models. R package version 3.1-151. URL: CRAN.R-project.org/package=nlme.
R Core Team, 2019. R: a language and environment for statistical computing. R Foundation for Statistical Computing v. 3.6.0, Vienna, Austria.
Rasmussen, J.J., Monberg, R.J., Baattrup-Pedersen, A., Cedergreen, N., Wiberg-Larsen, P., Strobel, B., Kronvang, B., 2012a. Effects of a triazole fungicide and a pyrethroid insecticide on the decomposition of leaves in the presence or absence of macroinvertebrate shredders. AquaticToxicology 118–119, 54–61.
Rasmussen, J.J., Reiler, E.M., Carazo, E., Matarrita, J., Munoz, A., Cedergreen, N., 2016. Influence of rice field agrochemicals on the ecological status of a tropical stream. Sci. Total Environ. 542, 12–21.
Rasmussen, J.J., Wiberg-Larsen, P., Baattrup-Pedersen, A., Monberg, R.J., Kronvang, B., 2012b. Impacts of pesticides and natural stressors on leaf litter decomposition in agricultural streams. Sci. Total Environ. 416, 148–155.
Reid, A.J., Carlson, A.K., Creed, I.F., Eliason, E.J., Gell, P.A., Johnson, P.T.J., Kidd, K.A., MacCormack, T.J., Olden, J.D., Ormerod, S.J., Smol, J.P., Taylor, W.W., Tockner, K., Vermaire, J.C., Dudgeon, D., Cooke, S.J., 2018. Emerging threats and persistent conservation challenges for freshwater biodiversity. Biol Rev Camb Philos Soc.
Rincón, J., Merchán, D., Sparer, A., Rojas, D., Zarate, E., 2017. La descomposición de la hojarasca como herramienta para evaluar la integridad funcional de ríos altoandinos del sur del Ecuador. Rev. Biol. Trop. 65, 321–334.
Santschi, F., Gounand, I., Harvey, E., A'lternatt, F., 2017. Leaf litter diversity and structure of microbial decomposer communities modulate litter decomposition in aquatic systems. Functional Ecology.
Schafer, R.B., Caquet, T., Siimes, K., Mueller, R., Lagadic, L., Liess, M., 2007. Effects of pesticides on community structure and ecosystem functions in agricultural streams of three biogeographical regions in Europe. Sci. Total Environ. 382, 272–285.
Schreiner, V.C., Feckler, A., Fernandez, D., Frisch, K., Munoz, K., Szocs, E., Zubrod, J.P., Bundschuh, M., Rasmussen, J.J., Kefford, B.J., Axelsen, J., Cedergreen, N., Schafer, R.B., 2018. Similar recovery time of microbial functions from fungicide stress across biogeographical regions. Sci. Rep. 8, 17021.
Sumon, K.A., Rico, A., Ter Horst, M.M.S., Van den Brink, P.J., Haque, M.M., Rashid, H., 2016. Risk assessment of pesticides used in rice-prawn concurrent systems in Bangladesh. Sci. Total Environ. 568, 498–506.
Swift, M.C., Cummins, K.W., Smucker, R.A., 1988. Effects of dimilin® on freshwater litter decomposition. Environ. Toxicol. Chem. 7, 161–166.
Thompson, P.L., MacLennan, M.M., Vinebrooke, R.D., 2018. Species interactions cause non-additive effects of multiple environmental stressors on communities. Ecosphere 9, e02518.
Tonin, A.M., Pozo, J., Monroy, S., Basaguren, A., Pérez, J., Goncalves Jr., J.F., Pearson, R., Cardinale, B.J., Boyero, L., 2018. Interactions between large and small detritivores influence how biodiversity impacts litter decomposition. J. Anim. Ecol. 87, 1465–1474.
Treton, C., Chauvet, E., Charcosset, J.-Y., 2004. Competitive interaction between two aquatic hyphomycete species and increase in leaf litter breakdown. Microb. Ecol. 48, 439–446.
Trimble, A.J., Lydy, M.J., 2006. Effects of triazine herbicides on organophosphate insecticide toxicity in *Hyalella azteca*. Arch. Environ. Contam. Toxicol. 51, 29–34.
Van den Berg, S.J.P., Baveco, H., Butler, E., De Laender, F., Focks, A., Franco, A., Rendal, C., Van den Brink, P.J., 2019. Modeling the sensitivity of aquatic macroinvertebrates to chemicals using traits. Environ. Sci. Technol. 53, 6025–6034.
Vinebrooke, R.D., Cottingham, K.L., Norberg, J., Scheffer, M., Dodson, S.I., Maberly, S.C., Sommer, U., 2004. Impacts of multiple stressors on biodiversity and ecosystem functioning: the role of species co-tolerance. Oikos 104, 451–457.
Von Fumetti, S., Blaurock, K., 2018. Effects of the herbicide Roundup® on the metabolic activity of Gammarus fossarum Koch, 1836 (Crustacea; Amphipoda). Ecotoxicology 27, 1249–1260. https://doi.org/10.1007/s10646-018-1978-5.
Wogram, J., Liess, M., 2001. Rank ordering of macroinvertebrate species sensitivity to toxic compounds by comparison with that of *Daphnia magna*. Bull. Environ. Contam. Toxicol. 67, 360–367.
Young, R.G., Matthaei, C.D., Townsend, C.R., 2008. Organic matter breakdown and ecosystem metabolism: functional indicators for assessing river ecosystem health Journal of the North American Benthological Society 27, 605–625.
Zubrod, J.P., Baudy, P., Schulz, R., Bundschuh, M., 2014. Effects of current-use fungicides and their mixtures on the feeding and survival of the key shredder Gammarus fossarum. Aquat. Toxicol. 150, 133–143.
Zubrod, J.P., Englert, D., Wolfram, J., Rosenfeldt, R.R., Feckler, A., Bundschuh, R., Seitz, F., Konschak, M., Baudy, P., Luderwald, S., Fink, P., Lorke, A., Schulz, R., Bundschuh, M., 2017. Long-term effects of fungicides on leaf-associated microorganisms and shredder populations-an artificial stream study. Environ. Toxicol. Chem. 36, 2178–2189.
Zubrod, J.P., Englert, D., Wolfram, J., Wallace, D., Schnetzer, N., Baudy, P., Konschak, M., Schulz, R., Bundschuh, M., 2015. Waterborne toxicity and diet-related effects of fungicides in the key leaf shredder Gammarus fossarum (Crustacea: Amphipoda). Aquat. Toxicol. 169, 105–112.